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Simple Approaches to Fluorescence Lifetime Standards Using Dye-Quencher Pairs

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Introduction

Photoluminescence techniques are amongst the most widely used tools in the material and life sciences, with new and exciting applications continuously emerging. Advantages include their comparative ease of use, unique sensitivity, non-invasive character, and potential for multiplexing, remote sensing, and miniaturization [1-4]. Increasing applications of fluorescence techniques in the life sciences and emerging medical applications of fluorescence microscopic techniques including 1P and 2P fluorescence microscopy combined with fluorescence lifetime imaging (FLIM) in e.g. *in vivo* eye diagnostics boosted the demand for robust, easy-to-use, and reliable fluorescence standards to ensure the reliability and comparability of fluorescence data. This includes fluorescence standards for the consideration of instrument-specific spectral and intensity distortions of measured signals and instrument performance validation, fluorescence intensity standards for the quantification of measured intensities and for signal referencing, and lately, also fluorescence lifetime standards [1,4-8].

Methods

Time resolved fluorometry and 2P FLIM were used to study the fluorescence decay behavior of several xanthene dyes in the presence of different concentrations of the quenchers KI, $Cu(II)ClO_4$, triethylamine, and tryptophan in aqueous solution.

Results

The fluorescence decays obtained for five xanthene dyes in aqueous solution in the presence of different concentrations of four quenchers were compared focusing on the size of the resulting lifetimes / realizable degree of quenching and the decay kinetics. With a fluorescence lifetime of 4 ns and monoexponential decay kinetics without quencher and quencher-tunable lifetimes between 4 ns and 0.2 ns, the pair rhodamine 6G-KI in PBS revealed the most intriguing properties.

Conclusion

We identified a first fluorophore-quencher pair as lifetime standard candidate. Ongoing studies focus on the ease of use of this approach under routine measurement conditions and the thermal and photochemical stability of this system and its reproducibility.

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